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Project leader:	Paul Lansell, Fera Science Ltd
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The results and conclusions in this report are based on an investigation conducted over a one-year period. The conditions under which the experiments were carried out and the results have been reported in detail and with accuracy. However, because of the biological nature of the work it must be borne in mind that different circumstances and conditions could produce different results. Therefore, care must be taken with interpretation of the results, especially if they are used as the basis for commercial product recommendations.

AUTHENTICATION

We declare that this work was done under our supervision according to the procedures described herein and that the report represents a true and accurate record of the results obtained.

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GROWER SUMMARY

Headline

Currently there is insufficient data to complete the evaluation of whether impaction spore samplers could be used as a lower cost method for spore trapping, reducing equipment costs and time in kit maintenance and sample processing. Further work in the final year of the project aims to secure enough data to complete the evaluation.

Based on ongoing evaluation work, there are several concerns around LFD being suited for testing spore samples collected with the alternative spore samplers.

Background

Downy mildew (*Peronospora destructor*) is a common disease that can result in major yield losses in bulb and salad onions and in onion seed production (Develash and Sugha, 1997) and crops may receive fungicide treatments as frequently as every 10 days. It is therefore crucial to have accurate and timely information around infection risk. Although the CropMonitor Pro (CMP) Platform by Fera Science Ltd on behalf of the Crop Health and Protection Centre now includes an onion downy mildew risk prediction module based on the MILLIONCAST algorithms (Gilles *et al*., 2004), this module cannot predict the onset of spore presence. This means that although CMP can help guide spray timings once spores are present, currently growers may use in-field spore sample detection methods to pinpoint the onset of the epidemic. This spore sampling is expensive and time intensive so any cost savings that can be made would enable increased uptake of these methods.

Summary

This project aims to validate the performance of two rotation impact samplers as a low-cost alternative to the more sophisticated suction traps for use in conjunction with a lateral flow device for detection of onion downy mildew (*Peronospora destructor*) spores to aid early detection of the pathogen entering onion crops. The three types of traps will be tested in onion trials at two locations provided by AHDB and/or G's. Further validation will be undertaken by growers evaluating the ease of use of the different samplers as decision aids in the field. Data collected will be used in modelling work to more clearly define spore thresholds for disease development in the crop. All trial sites will be hosted on an updated version of the CropMonitor Onion downy mildew module and results updated in real-time for use in local disease management decisions. Results at the AHDB and G's trials sites will be used for knowledge transfer activities to the wider industry, including presenting at relevant industry events (e.g. BOPA meetings; open days) across the 2022 season.

During the early stages of the project concerns were raised about the suitability of the MOLOGIC Lateral Flow Device (LFD) assay for detection of *P. destructor* spores collected using rotation impact samplers due to potential interference with the coatings (Vaseline or silicone grease) used on the spore collection sticks. This led to the project being extended to also validate the LFD for use in this setting whilst simultaneously developing Loop-Mediated Isothermal Amplification (LAMP) and real-time Polymerase Chain Reaction (PCR) assays, which could be used as alternative detection methods if the LFD is proven to not be fit for purpose.

The first sampling season provided limited sets of samples with which the sampler types could be directly compared. Further data is therefore needed to make any solid conclusions regarding potential differences in sampler type performance. A main concern is that in some cases the difference in spore numbers between replicates of the same impaction sampler type was found to be larger than the between sampler type difference. This could be a result of either inconsistencies in the sample collection or inability of the LFD to provide a quantitative measure of spore presence.

Initial validation has raised some concerns around the validity of the LFD to detect spore loads in samples collected with rotation impact samplers. This work needs to be completed before the start of the 2022 growing season. If the LFD shows to not be fit for purpose a further detailed comparison between the sampler types needs to be performed with the most appropriate diagnostic method.

Some concerns were also raised by the lab staff around the user friendliness of the LFD and the growers around operating the samplers. For example, the LFD is provided as a dipstick rather than a fully housed test kit. This means that the conjugate disc needs to be added by the user which risks cross contamination after which the stick needs to be dipped into the sample tube. Given that the tubes also contain ball bearings to get the Vaseline/Silicone coating which contains the spores off the matchsticks, it is difficult to add the LFD to the test tube without having lots of sample material sticking to the side of the LFD. Further feedback from growers on the experience setting up and operating the samplers was gathered following the end of the sampling season.

This first round of feedback will be used to adjust the 2021/2022 sampling protocols. Additional feedback around usability of the diagnostic methods and spore samplers will be collected through engagement with growers at events in 2022.

Financial Benefits

The exemplar sampler which is the Burkard cyclone spore trap has a price point of £2,547 excl. VAT (excluding batteries), whereas the alternative samplers have a much lower price point with the SporeStick coming in at £600 excl. VAT (excluding batteries) and the GRIPS-99M coming in at £525 (+ shipping & excluding batteries).

Not only are these alternative samplers cheaper to purchase they are believed to be easier to handle given that the weekly changeover of eight Eppendorf tubes in the Burkhard cyclone would be replaced by changing over two matchsticks (or plastic sticks) coated in grease, resulting in a significant weekly time saving (approximately 20 minutes time saving per changeover event per trap).

Action Points

- Completion of the assessment of the LFD as a suitable diagnostic method to be used in conjunction with the lower cost spore samplers
- A more detailed Fera-based comparison in performance of three sampler types using the diagnostic method identified as most appropriate for these traps
- Knowledge transfer event at one of the AHDB monitoring farms to test usability of the different sampler types and diagnostic methods (LFD and LAMP/PCR)

SCIENCE SECTION

Introduction

Downy mildew (*Peronospora destructor*) is a common disease that can result in major yield losses in bulb and salad onions and in onion seed production (Develash and Sugha, 1997) and crops may receive fungicide treatments as frequently as every 10 days. It is therefore crucial to have accurate and timely information around infection risk. Although the CropMonitor Pro (CMP) Platform by Fera Science Ltd on behalf of the Crop Health and Protection Centre now includes an onion downy mildew risk prediction module based on the MILLIONCAST algorithms (Gilles *et al*., 2004), this module cannot predict the onset of spore presence. This means that although CMP can help guide spray timings once spores are present, currently growers may use in-field spore sample detection methods to pinpoint the onset of the epidemic. This spore sampling is expensive and time intensive so any cost savings that can be made would enable increased uptake of these methods.

Here we investigate the potential to replace the exemplar Burkard cyclone spore trap with a lower cost alternative sampler. This project aims to validate the performance of two rotation impact samplers as a low-cost alternative to the more sophisticated suction traps for use in conjunction with a lateral flow device for detection of onion downy mildew (*Peronospora destructor*) spores to aid early detection of the pathogen entering onion crops. The three types of traps were tested in onion trials at two locations provided by AHDB and/or G's. Further validation will be undertaken by growers evaluating the ease of use of the different samplers as decision aids in the field. Data collected will be used in modelling work to more clearly define spore thresholds for disease development in the crop. All trial sites will be hosted on an updated version of the CropMonitor Onion downy mildew module and results updated in real-time for use in local disease management decisions. Results at the AHDB and G's trials sites will be used for knowledge transfer activities to the wider industry.

Main objectives

1. Validate rotation impact samplers for use as a more cost-effective alternative to suction samplers for robust detection of spore loads of *P. destructor* significant for increased risk to bulb and salad onion crops
2. Define spore thresholds for improved spray decisions for disease management in the crop
3. Deliver knowledge transfer to industry through demonstration of efficacy and value of an integrated decision support system for onion downy mildew.

During the early stages of the project concerns were raised about the suitability of the MOLOGIC Lateral Flow Device (LFD) assay for detection of *P. destructor* spores collected using rotation impact samplers due to potential interference with the coatings (Vaseline or silicone grease) used on the spore collection sticks. This led to the project being extended to also validate the LFD for use in this setting whilst simultaneously developing Loop-Mediated Isothermal Amplification (LAMP) and real-time Polymerase Chain Reaction (PCR) assays, which could be used as alternative detection methods if the LFD is proven to not be fit for purpose.

Materials and methods

Validation of suitability of the MOLOGIC LFD assay for detection of *P. destructor* spores collected using rotation impact samplers

Description of work delays

This work was originally scheduled to be completed by the end of January 2021 such that the outcomes of the validation could inform the choice of diagnostic test to be used for sample analysis of spore samples collected during the 2021 growing season. Several attempts were made to collect infected onion material to allow for the collection of live *P. destructor* spores and subsequent inoculum bulking for the validation work during late 2020. Unfortunately, there was limited infected material to work with and the inoculum bulking was unsuccessful. The continued lack of availability of live *P. destructor* spores hence delayed the start of the LFD validation work and LAMP and qPCR assay testing until August 2021.

Inoculum bulking and spore sample preparation

Onion bulbs were grown up in Fera glasshouses to provide vegetative material for inoculation using *P. destructor* spores sent to Fera in July 2021. Due to the sample being sent by post, the material was in a poor condition by the time it was received. Spores were washed from leaves showing the freshest looking sporulation and applied using a handheld atomiser to clean plants. Plants were sealed in plastic bags and incubated at 16 or 10°C in the dark for 48 hours. Following incubation plants were removed from the bag and kept at 16°C (16:8 light) and checked regularly for symptom development. Although leaf yellowing symptomatic of infection was seen, no sporulation developed when plants were wetted and placed in a sealed bag to increase humidity which should induce sporulation.

Further infected material was collected in person from Whitfields Eastwood Farm (CV32 6RA) in August 2021 and inoculation of clean plants repeated but this was also unsuccessful. Spore suspensions were prepared by washing spores off infected onion plants using distilled water and the spore concentration determined by counting all spores within 1 large square of a

haemocytometer (1 x 1mm). The spore suspension was frozen for use in future validation and quantification work.

For LFD validation, the volume of stock spore suspension needed for the required number of spores was calculated. Due to the Mologic protocol stipulating only a small amount (200µl) of buffer being added to the sample, we were concerned we would not be able to add large amounts of spore suspension for testing as this would overly dilute the buffer. This determined the maximum number of spores able to be tested. The spiking of matchsticks with predefined spore loads was done by dotting 10ul of spore suspension along the length of the stick and leaving them to dry overnight.

LFD performance validation

As an initial validation LFD tests were performed on a range of spore samples spiked into/onto different media, to test for potential inhibition/interference with the LFD test results from the media. The following were tested:

- 1,000 and 10,000 spores added to water
- 10,000 spores added to Vaseline or Silicone grease
- 10,000 spores spiked onto matchsticks coated with either Vaseline or Silicone grease
- Matchsticks coated with Vaseline or Silicone grease – no spores added

All tests were performed in triplicate.

Dilution series

Please note that the dilution series and exposure experiment have not yet been completed.

Dilution series, including a negative control, were prepared and tested using the LFD, PCR and LAMP method. The concentrations used were: 0, 50, 100, 500, 1,000, 5,000 and 10,000 spores. Each test was performed in triplicate. Dilutions series were repeated for spores being added to five different media: i) Vaseline, ii) Silicone grease, iii) matchsticks coated with Vaseline, iv) matchsticks coated with silicone grease and v) water.

The results for the dilution series whereby spores are added to water will be compared against the calibrated MOLOGIC dose-response curve (see Appendix A).

Exposure experiment

An exposure experiment was performed testing spore samples using the LFD only. There were again seven concentrations (0, 50, 100, 500, 1,000, 5,000 and 10,000 spores) with each test performed in triplicate. Spores were added to matchstick coated with Vaseline or Silicone

grease; whichever medium performed best under the dilution series work described above. The dilution series was then repeated for spiked matchsticks which were place on an active SporeStick sampler for 1, 4 or 7 days before testing with the LFD.

This experiment will give an indication of how sensitive the spores are to degradation whilst being exposed to e.g. UV light under field conditions.

Design of real-time PCR and LAMP assays for the detection of *P. destructor*

Internal Transcriber Sequences (ITS) of *P. destructor* and sequences of closely related fungal plant pathogens were obtained from GenBank and alignments produced. Species specific real-time PCR and LAMP assays were designed for *P. destructor* covering highly conserved regions. In total three real-time PCR and two LAMP assays have been designed and are awaiting validation for sensitivity and specificity.

Table 1. Real-time PCR assays designed for the detection of *P. destructor*

Sequence	**Primer**	**Melting temperature (°C)**	**Dye**
ACTTGGCGGCTGCTGGTGGCATG	Probe_1	64.1	FAM/TAM
CACGTGAACCGTATCAACCCAATTAATTGG	F_1	59.7	
ACTAGCTCCAACCGAGGTCAGAACA	R_1	59.97	
TATCAATGGAGGAGTGTTCGATTCGCGGTATGA	Probe_2	64.8	FAM/TAM
CTGCTGGTTGTGAAGGCTGTCAG	F_2	59.3	
AAACAGGCGCTTATTGAACGTTCTTCC	R_2	60.0	
TTTACTTGGCGGCTGC	Probe_3	68	MGB
GTGAACCGTATCAACCCAATTAATT	F_3	58	
TGATAGGGTTCGCTCAGCAGTA	R_3	59	

Table 2. LAMP assays designed for the detection of *P. destructor*

Sequence	**Primer**	**Melting temperature (°C)**
AAGCTACTAGCTCCAACCGAGGTCAGAACA	F1_1	64

GCCATGATAGGGTTCGCTCAGC	F-loop_1	58.6
ACTTGGCGGCTGCTGGTGG	F2_1	58.6
CACGTGAACCGTATCAACCCAATTAATTGG	F3_1	59.7
ATTGTAAACCCATTCTTAAATACTGATTATACTGTGGGGACG	B1_1	63.7
ACTAGATAGCAACTTTCAGCAGTGGAT	B-loop_1	58.9
CGTTCTTCATCGATGTGCGAGC	B2_1	58.8
CTGAATCCTGCAATTCGCATTACGTATC	B3_1	60.08
CATACATTTCAAAGGACTCACAGCCGATCCG	F1_2	63.7
CAGCCGCAAGACACTTCACATCT	F-loop_2	59
CTTCTTTCCGTGTAGTCGGTGGAGGA	F2_2	60
CCTGGGAGTATGCCTGTATCAGTGTCC	F3_2	60.6
GAAAAGCGTGGCGCTGCTGGTTGTGA	B1_2	65
TCAGTATGGCTAGTCGGCGACC	B-loop_2	58.6
CTCCTCCATTGATACCATAGCAGACA	B2_2	58.1
GCCTGTTTAGCCGAAGCCAATCATAC	B3_2	60

Field trials to compare the performance of rotation impact and suction spore samplers

Description of changes to original project

Because of the concerns around the suitability of the LFD for detection of *P. destructor* spores collected using rotation impact samplers and the delays with the LFD validation work until after the start of the 2021 field season, in 2021, only a limited number of samples were analysed. This allowed for i) an initial assessment of whether the LFD test results are affected by the spore trap type with which the samples were collected, ii) some within season information to be provided to the site managers to assist disease management and iii) initial testing of the usability of the data provided. At the same time the limited testing with the LFD meant that enough samples would be retained for retrospective testing with the method (LFD / LAMP / PCR) which proves most suitable for the validation of the spore traps.

Covid-19 also reduced opportunities for direct interaction with growers and the associated uncertainties around field access, potential job losses and other economic impacts meant Fera was unable to find two independent growers to volunteer for the in-field sample testing.

The in-field testing was therefore cancelled for the 2021 growing season. However, G's kindly offered an additional site for sample collection, leading to five sites in total, rather than the original four sample collection and two in-field testing sites.

Field trial sites and sample collection

Year 1 (2021): Experimental work to validate spore traps was undertaken at five sites provided and managed by G's with sites located at: i) Woodhall in Shropshire, ii) Luddington in Warwickshire, iii) Hainey in Cambridgeshire, iv) Norfolk and v) Tunstall in Staffordshire. At each site two different types of rotation impact sampler were tested alongside a Burkard cyclone suction sampler. Sampling commenced during the beginning of April through to late July with spore samples collected twice a week until spores were detected with the LFD at which point samples were to be collected weekly until symptoms are observed at the site at which point spore sampling ceased.

Spore samplers

Three types of spore traps were used:

Standard rotation impact samplers. A **Rotorod® Model 20** (Sampling Technologies INC., Minnetonka, MN, USA) or a **GRIPS-99M** (Aerobiology Research Laboratories, Ottawa, Ontario, Canada) was placed at each trial location apart from the Tunstall site. These are basic rotation impact samplers which consist of two non-retractable plastic rods (<2 mm wide) which are attached to a bar which spins at a constant 2400 rpm driven by a motor attached to a 12 V battery. The rods were coated in an embedding grease (vaseline) which results in spores being impacted onto the greased surface of the leading edge of the rod when the device spins. Sampling occurs independently of wind direction, so the trap did not need specialist positioning. There is no timer incorporated, so the devices run consistently during the sampling period or must be manually switched on and off by the user at the appropriate times. Experience with these devices shows that they should easily run for a week in the field without need to change the battery (12V DC). The price point for these traps is $900 CAD (+ shipping) for the GRIPS-99M (excluding batteries).

New generation impact sampler. Launched in March 2019, the **SporeStick** (Optisense, UK) has been developed in collaboration with aerobiology experts at Fera Science Limited to address the need for a versatile, low cost spore sampling device for detection of fungal spores. The SporeStick is similar in design to the two standard rotation impact samplers, with a rotating bar and two sampling rods coated in grease. These rods are also non-retracting, as research at Fera showed that retraction causes contamination of the device, which can be transferred when new rods are inserted. Instead of plastic rods the SporeStick uses wooden sticks, which are commercially available and more environmentally friendly. The wooden

sticks have also been found to be more efficient in spore capture than the plastic rods (Chandelier et al., 2014) due to their rough surface. The SporeStick also incorporates a timer so that the device can be operated at specific periods of day or night or intermittently to prolong sampling efficiency (e.g. 1 min in every 10 minutes). The motor has a variable speed option and a forward and reverse function allowing the device to sample spores onto both the front and rear surfaces of the stick hence doubling the sampling surface and prolonging operational efficiency. Calibration equations used to calculate the equivalent number of spores per cubic metre of air have been adjusted accordingly to use data from this device. Finally, the device has an app https://appadvice.com/app/sporestick/1456794256). which allows the user to programme the device speed of rotation and direction and set bespoke rotating periods. The price point for this trap is £600 excl. VAT (excluding batteries).

Suction sampler (Burkard cyclone spore trap). Previous AHDB-funded research on spore trapping of P. destructor has used a **Burkard** MTIST sampler or a Burkard cyclone sampler and so these two devices have already been validated for use in trapping spores of *P. destructor*. It is proposed to use the Burkard cyclone sampler as the exemplar suction spore trap for this project as it is the most widely used and is available commercially. In addition, Fera has access to a significant number of these devices and considerable experience with operating them over many seasons. The Burkard cyclone trap samples at a flow rate of 16.6 L min^{-1} into a 1.5mL tube which is mounted in a rotating carousel. The dry cyclone impacts particles into the tube for a user-defined period of time (usually 24 hrs) after which the carousel turns to expose the next of up to seven tubes in total. The timing of capture/detection of the spores can therefore be more accurately determined using this device compared to the rotation impact sampler, but these devices are more labour intensive to set up and maintain and can be more prone to the effects of weather conditions. They are also more expensive with a price point of £2,547 excl. VAT (excluding batteries).

Quality assurance of trap performance prior to use. The specific set up and age of the devices can affect their trapping performance, so all rotating impact samplers were serviced and set to the standard operating speed of 2400 rpm using a tachometer. The flow rate of the suction traps was also calibrated to 16.6 L per minute prior to deployment. This is not a universal standard procedure for spore traps, but routine maintenance schedules need to be implemented if these devices are to be reliably operated on-farm as a decision support device. Research shows that traps can deviate from their original settings over time (Frenz & Elander, 1996) and this would affect their performance in delivering consistent data for risk prediction.

Sample testing for spore presence

Following the defined exposure period, the rods or microfuge tubes located at the five sampler validation sites were removed from the traps and sent to Fera using pre-paid envelopes and stored in a freezer until testing occurred. The growers were also asked to record the first date of symptom expression at the trial sites. The rotation impact devices generated two replicate samples per sampling period whereas the suction sampler generated seven daily samples per week.

Sample preparation. To capture seasonal trends and potentially define spore thresholds for infection, during the 2021 growing season two out of seven Burkard samples were analysed per week per site, with the two out of seven samples selected at random. Five drops of the LFD buffer were added to the Burkard sample tube after which the sample was vortexed.

To evaluate potential differences in spore trap type performance we also analysed all samples from several sample dates and sites covering samples from all trap types for which samples were returned. The most consistent 2021 samples (i.e. sample dates for which reliable samples were returned for multiple traps) were selected for this analysis (Table 3). Single Burkard samples were processed as outlined in the previous paragraph. When the content of multiple Burkard tubes needed to be combined the content of the first tube was emptied into to next tube and vortexed again at each step until the content of all tubes was combined with the original five drops of buffer. All previous tubes were then centrifuged for 10 seconds to collect any remaining buffer which was pipetted into the final tube.

Spore sticks were placed in transport tubes with three 5 mm ball bearings, five drops of buffer added and shaken by hand for 1 minute.

Spore presence testing by LFD. Testing for spore presence was done using the MOLOGIC LFD following the protocol defined in Appendix A. All LFD tests were read using an electronic cube reader to capture a digital measure of the result. The cube readers were calibrated to read the specific LFD for onion downy mildew. Calibration curves will be used to calculate the equivalent number of spores in each sample.

In-season results reporting. All four trial crops were set up on the onion downy mildew module of CropMonitor Pro (www.cropmonitor.co.uk) which displayed daily updates on predicted risk of sporulation and infection by *P. destructor* at the location. Results of the diagnostic tests performed at Fera were added to the system with the option of first symptom reports to be added directly to the database and displayed on the downy mildew risk pages.

Deliver knowledge transfer to industry through demonstration of efficacy and value of an integrated decision support system for onion downy mildew

Website enhancements for onion downy mildew module on CropMonitor Pro (CMP)

The AHDB CMP onion downy mildew module featuring the Milioncast model was extended to capture results from LFD tests and disease observations. The site managers for the five trial sites ran during the 2021 growing season were provided with access to the updated CMP services to support disease management.

User feedback questionnaires

During the first 2021 growing season and mainly due to covid-19 restrictions and uncertainties it was not possible to engage independent growers to collect user experience data as per the original proposal. Instead the number of sites run by G's was increased from four to five sites. Additionally, more detailed user feedback forms were created to capture the site manager's experience with:

1. Spore trap set-up
2. Spore trap maintenance
3. Sampling protocol and logistics
4. CMP data visualisation
5. Management and communications

See Appendix B for the full list of questions. The questionnaire results will be used to improve site management and user experience during the 2022 growing season. A similar survey will be performed after the second growing season to assess improvements made.

We have also collated feedback from the Fera lab-staff around the usability of the LFD.

Other

See Knowledge and Technology Transfer section for further details.

Results

Validation of suitability of the Mologic LFD for detection of *P. destructor* spores collected using rotation impact samplers

Figure 1 shows that the cube reading performed on LFD tests applied to spore samples created in different media is not affected by the media they were in. Matchsticks coated with either Vaseline or Silicone grease with no spores added gave a negative LFD result, indicating there was no interference by the products resulting in a false positive. Any low readings on the cube reader from Rotorod/Sporestick samples could therefore be confirmed as a genuine result. The results for the match sticks coated with Vaseline or Silicone grease are not shown as during the spiking with the spore solution the spiking droplet touched the tray and ran off the stick. It was therefore unclear how much of the original concentration was spiked onto the stick. In these cases, another droplet was added, with the LFD scores

suggesting that quite a lot of the spores within the original droplet had actually made it onto the stick. Because the spore concentration added is not clear and not the same as for the other validation samples the results for these samples were excluded. The protocols for spiking coated matchstick with spore solutions have now been fine-tuned.

The additional validation work including the dilution series for the LFD, PCR and LAMP and the LFD exposure experiment are still ongoing.

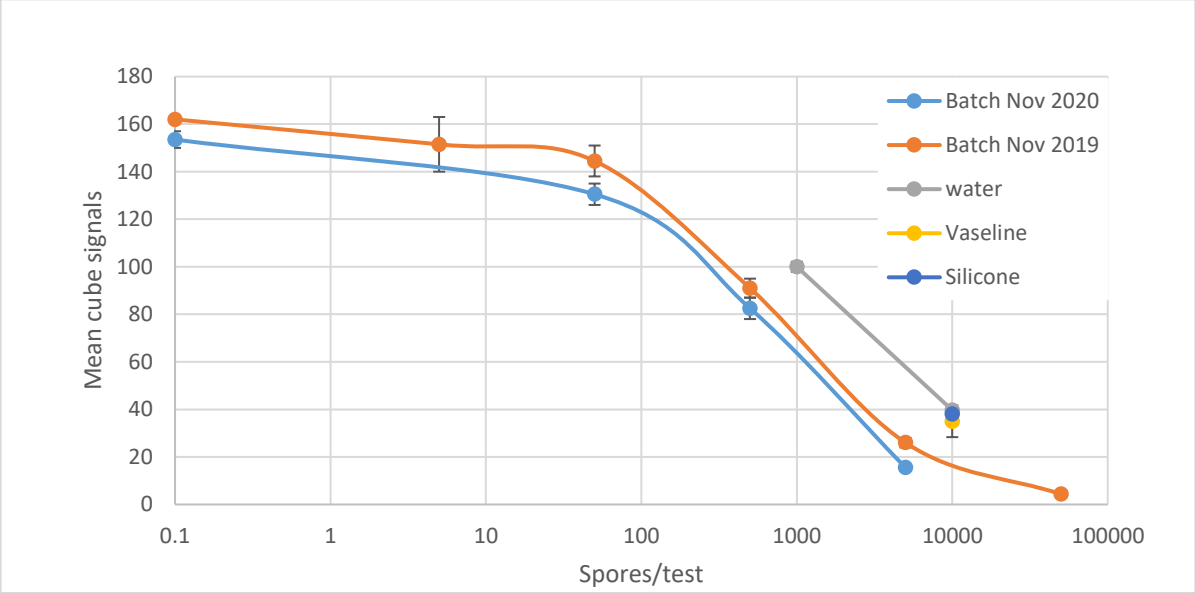


Figure 1. Mean cube signal versus spores per test for LFD tests performed on spore samples created in different media and compared to the MOLOGIC calibration curves for the 2020 (and 2019) batch.

Validation of rotation impact samplers for use as a more cost-effective alternative to suction samplers

2021 season

Table 3 shows that there are clear differences between the spore estimates depending on which type of sampler the spores have been collected with. However, there is no clear pattern in the data with the Burkard in one case finding significantly higher spore numbers than both impaction samplers (VCS Tunstall) whereas the opposite is true for the Luddington site (03/06-10/06). The difference in spore numbers between replicates of the same impaction sampler type is often larger than the between sampler type difference.

Table 3. Comparison of cube reading scores of the LFD results for each spore trap type and multiple site/dates. Each impaction sampler has two sample sticks and hence results are provided for both replicates/sample sticks.

Site/Sample period	Cube reading							
	SporeStick		Rotorod/GRIPS		Burkhard [1]			
	Rep 1	Rep 2	Rep 1	Rep 2	First single day	Second single day	5 days combined	7 days combined
Hainey 16/04-23/04	18	14	10	-	100 (16/04)	29 (21/04)	11	
Luddington 03/06-10/06			17	4.1	122 (04/06)	109 (07/06)	43	
Hainey 08/07-15/07	39	5.3 [2]	24	5.7			8	
VCS Norfolk 15/07-22/07	5.6 [2]	9.2	3.9 [2]	18			4.6	
Hainey 15/07-22/07	18	4	14	3			14	
Hainey 22/07-29/07	29	6.6	12	11				16
Hainey 29/07-05/08	5.3	26	22	10				11
Hainey 05/08-12/08	15	6.7	19	19				10
VCS Tunstall	49	73	83	92				11

05/08-12/08 [3]								
Hainey 12/08-19/08	0.7	20	10	7.7				6

[1] Up to the end of July two of the seven Burkard sample tubes were used each week for in-season testing with the LFD. For the spore trap performance comparison, the content of the remaining five sample tubes were combined and tested with a single LFD to provide a more direct comparison with the weekly spore stick samples. For the later samples the content of all seven Burkard sample tubes were combined and tested with single LFD.

[2] LFD didn't run properly; no visible control line.

[3] For this sample Sporesticks were placed in transport tube with 2.3mm zirconia/silica beads and shaken for approx. 1 min however a lot of material still visibly remained on the sticks, which may have resulted in less spores being recovered than was the case for the Burkard sample. The protocol was subsequently amended to use three 5mm ball bearings.

Define spore thresholds for improved spray decisions for disease management in the crop

In total 95 samples collected with the Burkard trap were analysed using an LFD. Cube reading for all were recorded and have been depicted in Figure 1. Very few samples (6 %) were suggesting the presence of spore loads greater than 5000 spores (cube readings which fall below the red line; cube reading < 15.5), whereas the LFD tests suggested that 49% of the sample tubes contained spore loads greater than 500 (cube reading < 82.5).

By Mid -August no disease had yet been reported at any of the sites, apart from the Luddington grower who at that point reported a high amount of sporulation being observed around the area. This limited information around symptom onset before the end of the season, made it impossible to establish a correlation between (total) spore load and symptom onset date, which would allow us to define a tentative field-based spore threshold.

Additionally, deviations from the protocol meant that twice weekly samples were not collected at any of the sites. However, Figure 1 suggests that spores were detected at all sites by the beginning of May at which point sample collection would have switched to a weekly collection and hence this suggests that the protocol deviations have not lead to a large reduction in total samples to be collected.

The data is suggestive of the spore loads being generally low before mid-April with more frequent ‘high’ spore loads from then on. Only two samples were received for the Woodhall site at the start of the growing season. For the VCS Norfolk site there appear to have been two periods of increased spore presence, one during mid-June and one at the end of July. For VCS Tunstall there also appear to have been two periods of higher spore loads, one during the end of May and early June and one during the end of July. At the Luddington site only a few medium spore loads were observed between mid-April and early May. The Hainey site by contrast was exposed to medium to high spore loads from early May onwards, with the highest spore loads observed during the end of May to mid-June and during mid to the end of July.

Figure 2 shows that infection risks as predicted by the CMP Millioncast model were generally low throughout the season for all sites, with only 4 moderate risk event predicted between mid-April and mid-August for the Luddington site and only a maximum of 15 moderate and 1 high risk event predicted during this whole period for the Norfolk and Tunstall site and 2 high risk, yet only 10 moderate risk events at Woodhall. Moreover, the periods of higher infection risk don’t seem to match the spore presence periods, which is most likely to explain the late appearance of disease symptoms.

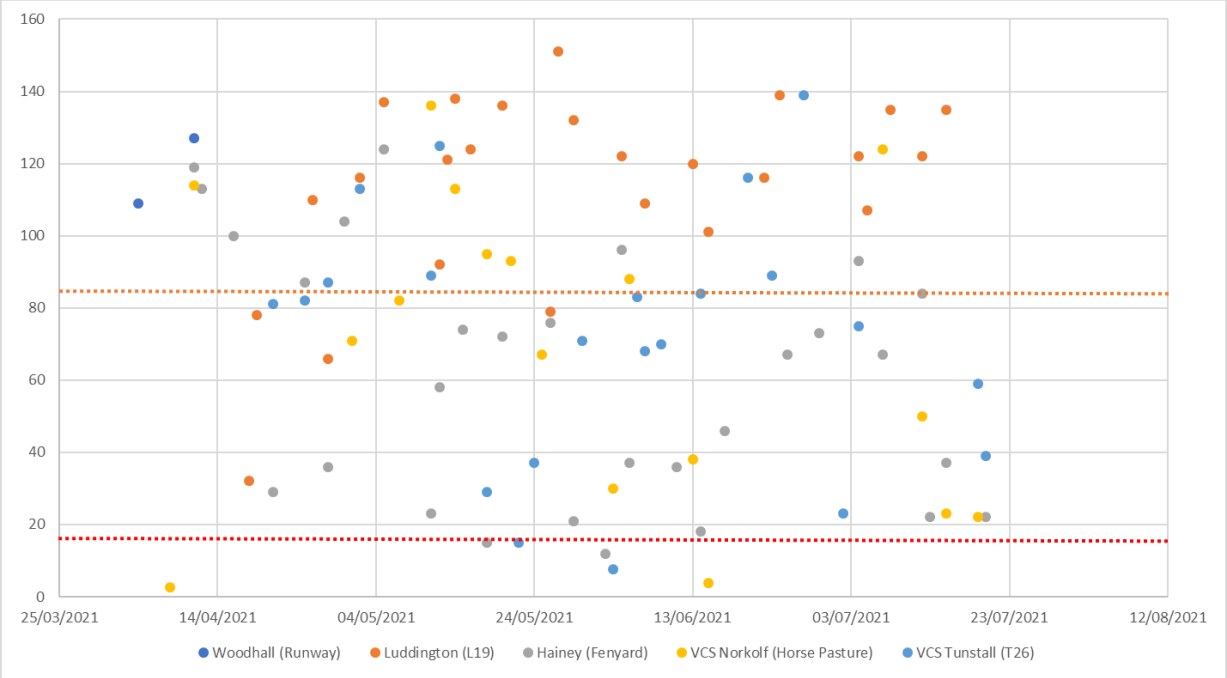


Figure 1. Cube reading values recorded after LFD was applied to spore samples collected at the five sampling sites during the 2021 growing season. The orange and red dotted lines indicate cube readings indicative of 500 or 5000 spores, respectively, as per the MOLOGIC LFD calibration curve (Figure A1 in Appendix A). Note that lower cube readings indicate higher spore loads.

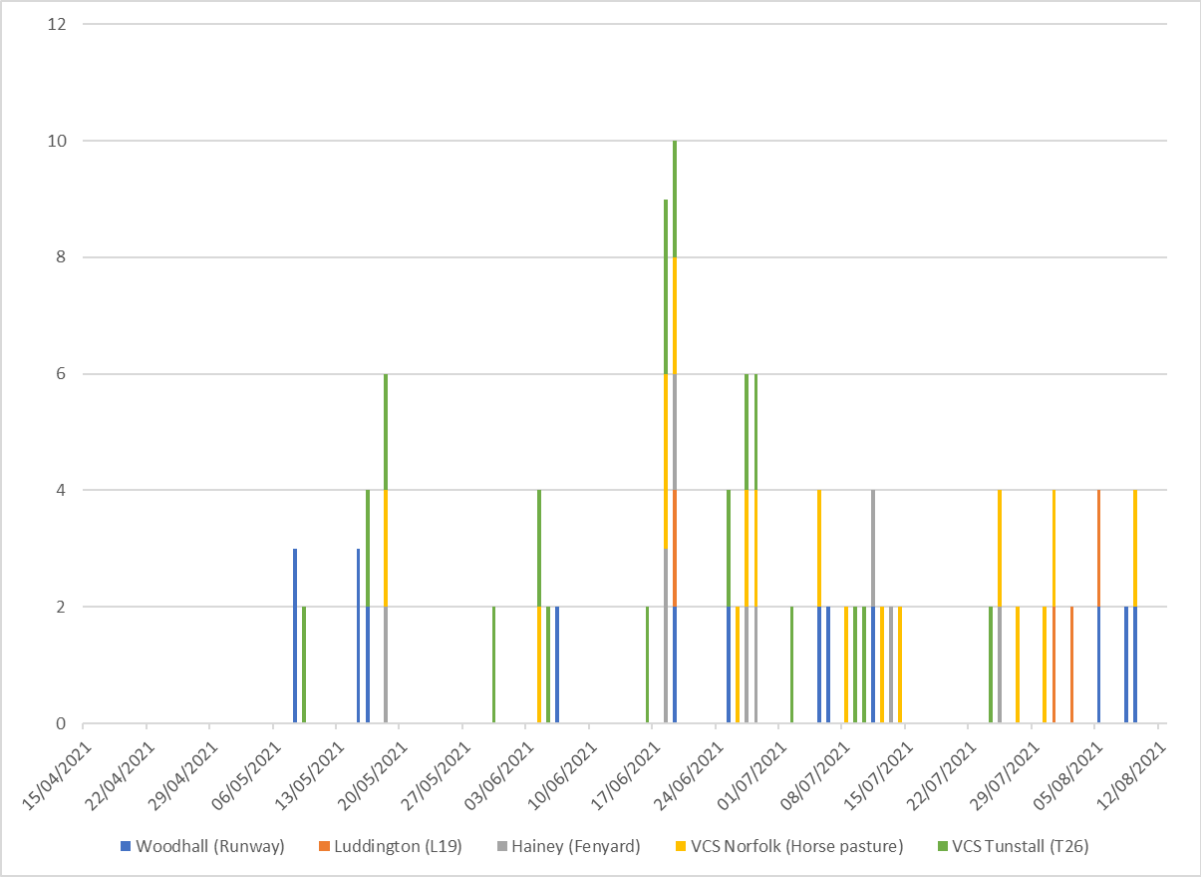


Figure 2. CMP risk of infection with moderate risks represented by bars of height 2; high risks represented by bars of height 3 and low risks excluded for clarity.

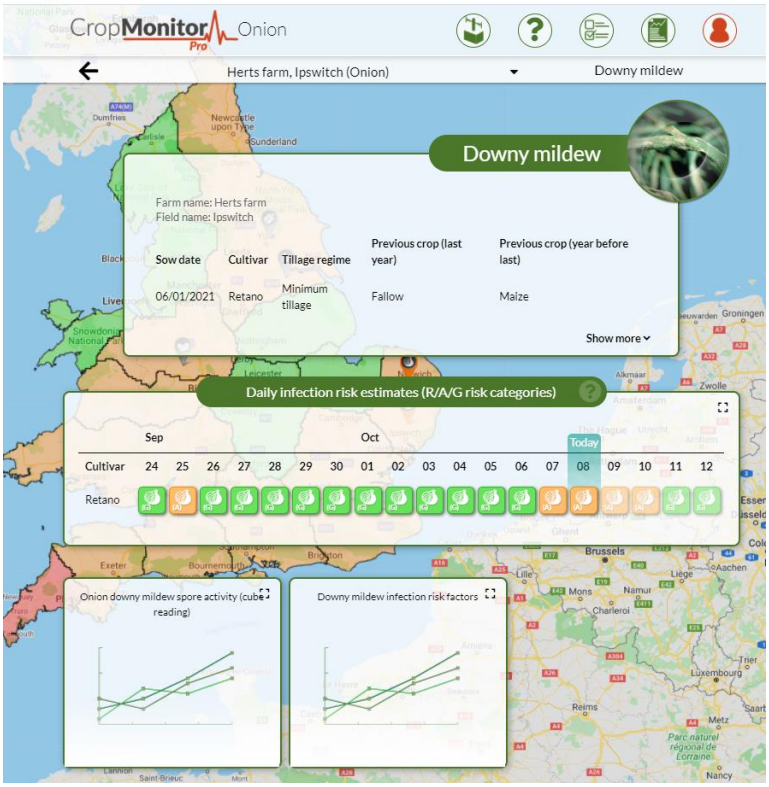
Deliver knowledge transfer to industry through demonstration of efficacy and value of an integrated decision support system for onion downy mildew

Website enhancements for onion downy mildew module on CMP

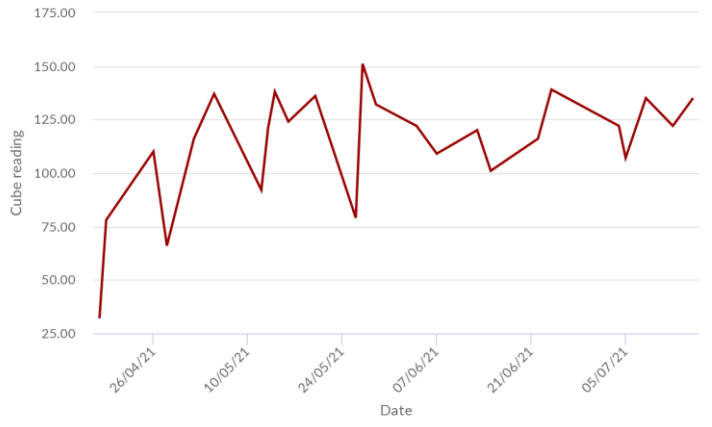
Two videos were generated and are now hosted on the AHDB horticulture website to 1) guide registration on CMP and add an onion field and 2) how to use the onion downy mildew risk prediction tool. Please see https://legacy.ahdbdigital.org.uk/onion-downy-mildew-risk-predictor for the videos.

Figure 3a illustrates the updated site (field) specific page of the CMP onion downy mildew module. It now includes a plot illustrating the cube readings submitted for specific LFD testing dates (Fig. 3b) in addition to the downy mildew infection risk factors plot (Fig. 3c). The data reflected within this plot can either be entered directly into the database by the Fera project team or can be added by the site manager through the site specific 'My Sightings' page they have access to through their CMP registration. Currently, users can only enter either cube readings or MOLOGIC lateral flow score card readings, with the latter ranging between 0 and 10. In future the data entry options can easily be extended to allow for PCR or LAMP test result entries.

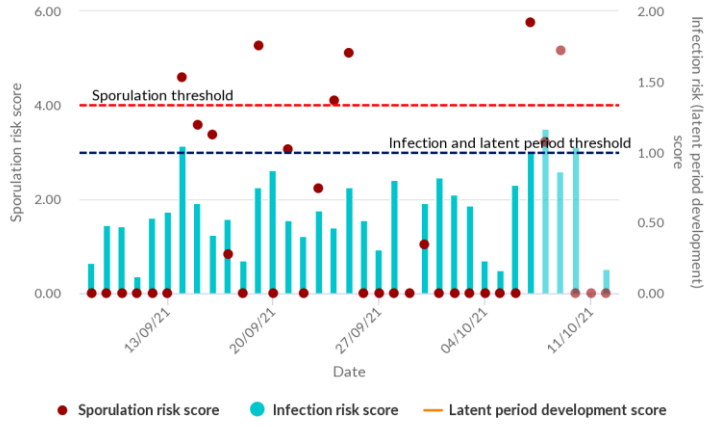
Figure 4 illustrates the new user data entry functionality available on the 'My Sightings' page. Users can submit both disease observation and spore presence test result data, with the spore presence test result data being visualised on the site-specific pages the day after data submission.



a



b



c

Figure 3. a) CMP onion downy mildew module site/field specific page, with expanded spore presence and infection risk factors plots in b) and c), respectively.

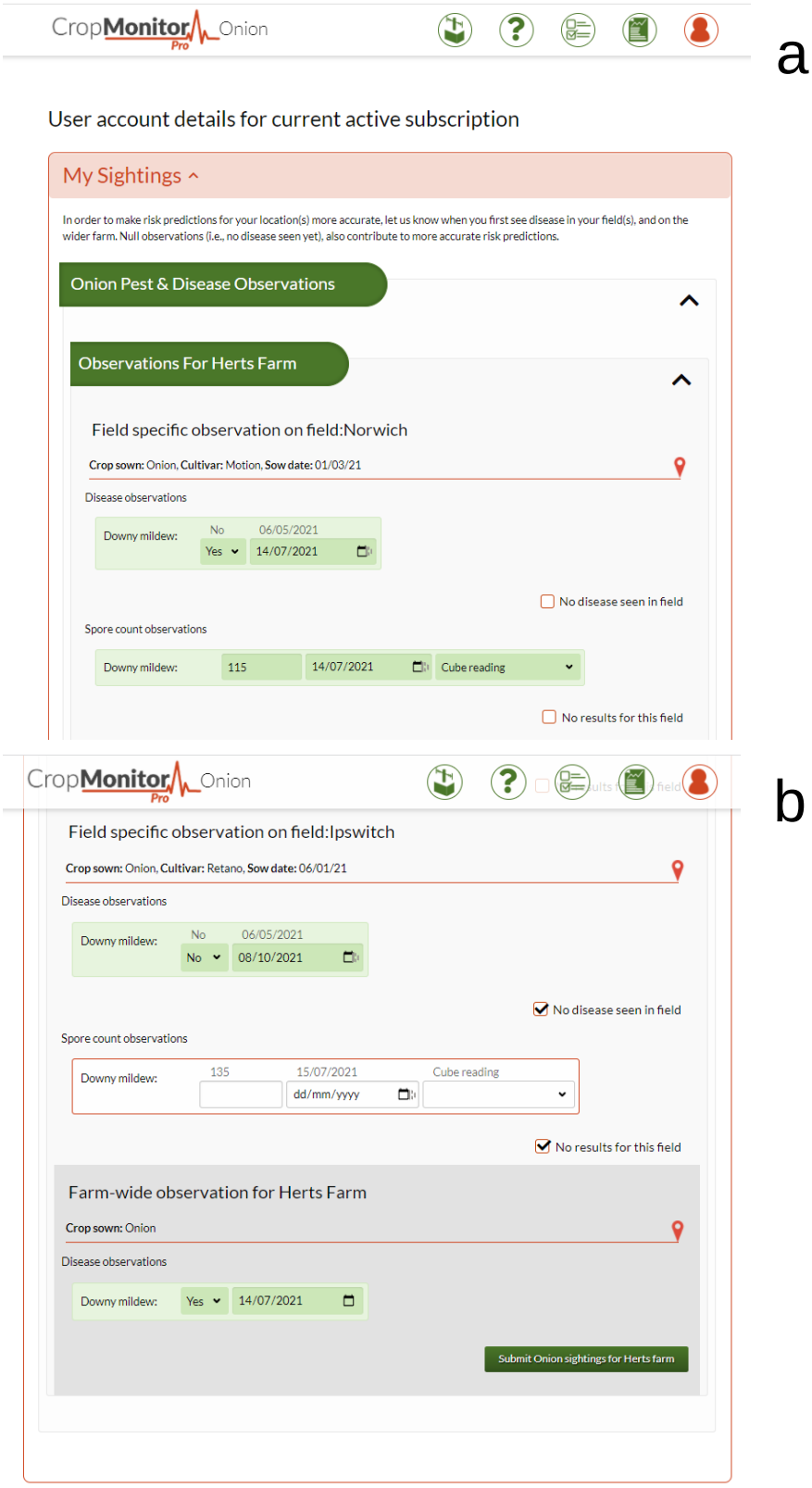


Figure 4. CMP data entry functionality for onion downy mildew as provided on the 'My Sightings' page. Data can be submitted per site (a) and/or for the whole farm (b).

User feedback questionnaires

All questions included in the 2021 user feedback questionnaire and the anonymised responses of the site managers are provided in Appendix B. Two out of four site managers have completed the questionnaire at the time of compiling this report. Experiences were varied with one grower generally more comfortable with the set-up, running and maintenance of the traps than the second grower. Overall, the rotorod and SporeStick were perceived easiest to set-up and allowed for ease sample changeover, but the Burkard trap was perceived most reliable. The SporeStick was scored very negatively with regards to battery life. One grower experienced only limited challenges with the collection of samples, whereas the other grower experienced time pressures and equipment failure, making sample collection a challenge.

Other

See Knowledge and Technology Transfer section for further details.

Discussion

The main aim of this study was to validate rotation impact samplers for use as a more cost-effective alternative to suction samplers for robust detection of spore loads of *P. destructor* significant for increased risk to bulb and salad onion crops. The first sampling season provided limited sets of samples with which the sampler types could be directly compared. Further data is therefore needed to make any solid conclusions regarding potential differences in sampler type performance. A main concern is that in some cases the difference in spore numbers between replicates of the same impaction sampler type was found to be larger than the between sampler type difference. This could be a result of either inconsistencies in the sample collection or inability of the LFD to provide a quantitative measure of spore presence.

In fact, the results derived during the study so far suggest that although the LFD appears to accurately detect spore presence, it may not be suitable for measuring spore pressures quantitatively. For example,

- The MOLOGIC calibration curve suggests you cannot accurately identify spore loads <50 (potentially closer to <100 as the next point on calibration line is 500 spores). This could be important if you want to identify early low spore loads or if we need to accurately determine an accumulative spore load and there are lots of days with low spore loads which would then significantly change the estimate for the onset date of the epidemic.
- For onion downy mildew the cube score decreases from ~160 (in the absence of spores) with increasing spore load, which is used to represent spore loads varying

between 0 and >50,000. This means that small differences in the cube reading lead to large difference in spore numbers. This is not too much of a concern for high spore loads, but we need to feel assured that readings at low spore concentrations are accurate.

- The variability of the cube reading scores between replicates is very high. In general, we only had samples from multiple traps from dates where disease pressures were high and in these cases it may not be essential for the tool to accurately predict spore loads. However, the LFD validation work has shown that in general the spore loads estimated using the cube reading and MOLOGIC calibration curve is significantly lower than the known concentration with which the samples were spiked.

The validation work to assess the validity of the LFD (& PCR & LAMP) to detect spore loads in samples collected with rotation impact samplers needs to be completed before the start of the 2022 growing season. If the LFD shows to not be fit for purpose, a further detailed comparison between the sampler types needs to be performed with the most appropriate diagnostic method.

Fera has so far been unable to replicate the MOLOGIC calibration curve as presented in Appendix A. Although our results found no effect of the media into which the spores were spiked on spore numbers detected by the LFD, the resultant dose-response curve was shifted to higher cube readings and hence lower spore numbers. The dose-response curve is however of the same shape. Potential reasons for not being able to replicate the MOLOGIC curve are:

- Spore counts were inaccurate leading to incorrect spore numbers being used to spike samples when creating the spore suspensions for the dilution series. When spores are washed off the solution is dirty and spores are hence hard to count. However, the counting was done by an expert and it is unlikely that the spore counts were as different as observed, i.e. 5000 instead of 10000.

- The LFDs were nearly a year old by the time the validation took place. The shelf-life may have been affected. Given that growers would be likely to buy enough LFDs to cover a full season and that the LFD costs significantly more when bought in low numbers the expectation is that that shelf-life would be at least a full year.

- The buffer may have degraded. The buffer was left outside the fridge due to a miscommunication by the sender. The buffer was eventually moved to the fridge and in the meantime was stored in a cool lab. The supplier was contacted before the buffer was used and they said this is unlikely to be an issue. This is unlikely to be the reason for the mismatch

as buffer degradation would likely have resulted in a much higher variability rather than a consistent shift in curve.

- The cube reader is incorrectly calibrated. This would result in a consistent difference as the one observed here.

Some of these factors may raise concerns around whether the LFD is fit for purpose. The validation work will be repeated with new LFDs and fresh spore solutions to exclude some of the above.

Some concerns were raised by the lab staff around the user friendliness of the LFD and the growers around the samplers. The LFD is provided as a dipstick rather than a fully housed test kit. This means that the conjugate disc needs to be added by the user which risks cross contamination after which the stick needs to be dipped into the sample tube. Given that the tubes also contain ball bearings to get the Vaseline/Silicone coating which contains the spores off the matchsticks, it is difficult to add the LFD to the test tube without having lots of sample material sticking to the side of the LFD. In addition, lab staff reported that when LFDs were read a second time, a few minutes after the 15 minutes as defined in the protocol had expired, in several cases the treatment reading had changed by a few units. It needs to be confirmed whether this also happens when spore densities are low, given that if the time till testing needs to be this accurate then this raises concerns about the usability of the LFD in the field by growers who are unlikely to read the LFD after exactly 15 minutes.

The grower feedback highlighted that although the impaction samplers were perceived easier with regards to set-up and sample changeover as compared to the Burkard trap, the limited battery life is a major concern. Battery life issues could be overcome for some growers by manually connecting the sampler and battery to a solar panel, which extended the battery life of the devices and removed this limitation. The SporeStick uses bespoke batteries and Fera will contact the supplier to raise these concerns.

Knowledge and Technology Transfer

The main report describes website enhancements made to the onion downy mildew module on CMP and the user questionnaires developed to assess the grower's experience with using the different types of spore traps.

Fera will attend CropTec 2021 during 24 and 25 November and will be talking amongst other things about their decision support development, spore trapping and diagnostic testing expertise. When applicable Fera will engage with onion growers to capture their views on the developments and current achievements from this project. Specific outcomes of such

discussions (if they arise) will be reported verbally during the next project meeting and will also be captured in the final report.

Project outcomes were disseminated at the June 2020 and June 2021 AHDB progress meetings.

No other events have taken place due to Covid-19 restrictions, which also meant that the on-farm demonstrations could not take place.

Appendices

Appendix A. MOLOGIC Onion Downy Mildew Assay Method

- Invert dropper bottle and add 5 ‘hanging’ drops (200µL) of sample buffer to sample tube.
- Leave for 30mins on the bench
- Add 1 conjugate disc to the sample tube and mix with sample buffer by flicking bottom of tube for 10 secs. Leave for 5 minutes and mix again for 5 secs.
- Add test strip into sample with red ‘hold tab’ at the top.
- Leave strip to run for 15 minutes.
- Remove strip from sample and place in cube holder with base outside of reader and top butted at end of strip holder (refer to picture). Close holder and read (as per SOP197).
- Record T (test Line) reading and check for visible control spot/line.

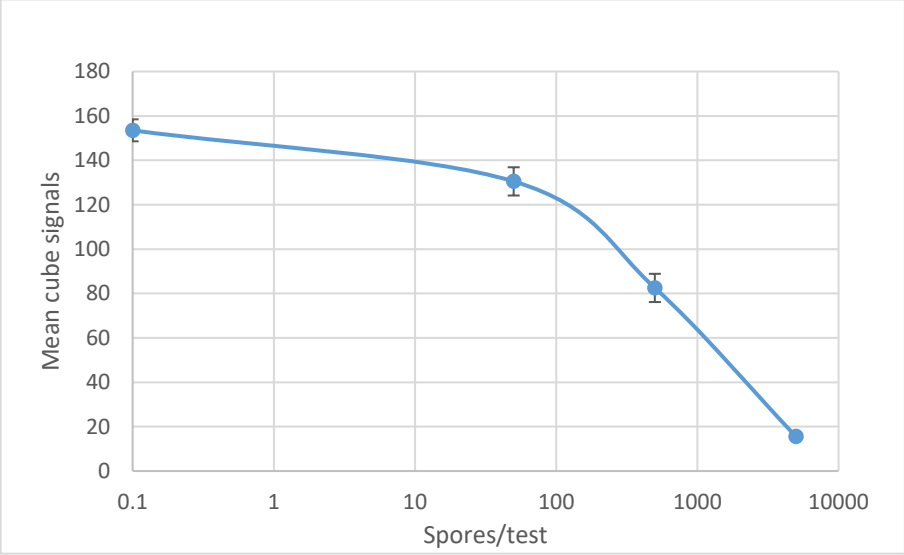


Figure A1. Typical MOLOGIC onion downy mildew LFD assay performance

Appendix B. Grower feedback

This Appendix list the questions included in the grower feedback questionnaire and the responses provided. The questionnaire was sent to the four growers running the five sites and the AHDB project officer. Only two responses were received.

1. Thinking about setting up the spore traps, please rate each device for ease of setting up
    a. Burkard spore trap:
        i. Easy: 1
        ii. Difficult: 1
    b. Rotorod
        i. Easy: 2
    c. SporeStick
        i. Easy: 1
        ii. Difficult: 1
    d. Please use this space to provide any additional feedback/details on challenges faced setting up the spore traps
        i. Spore stick battery did not power the trap. We resorted to using alternative batteries and adapting wires.
2. Thinking about maintaining the Burkard Spore Trap, please rate the device's performance against each category
    a. Lifespan of batteries:
        i. Good / Easy: 2
    b. Reliability of device
        i. Good / Easy: 1
        ii. Very good / Very easy: 1
    c. Sample changeover
        i. Good / Easy: 2
    d. Ease of operation
        i. Good / Easy: 2
    e. Robustness of device
        i. Good / Easy: 1
        ii. Very good / Very easy: 1
3. Thinking about maintaining the Rotorod Spore Trap, please rate the device's performance against each category
    a. Lifespan of batteries:
        i. Poor / Difficult: 1
        ii. Good / Easy: 1

b. Reliability of device
    i. Poor / Difficult: 1
    ii. Good / Easy: 1
c. Sample changeover
    i. Good / Easy: 2
d. Ease of operation
    i. Good / Easy: 1
    ii. Very good / Very easy: 1
e. Robustness of device
    i. Very poor / Very difficult: 1
    ii. Good / Easy: 1

4. Thinking about maintaining the SporeStick Spore Trap, please rate the device's performance against each category
    a. Lifespan of batteries:
        i. Very poor / very difficult: 1
        ii. Poor / Difficult: 1
    b. Reliability of device
        i. Poor / Difficult: 1
        ii. Good / Easy: 1
    c. Sample changeover
        i. Good / Easy: 1
        ii. Very good / Very easy
    d. Ease of operation
        i. Poor / Difficult: 1
        ii. Good / Easy: 1
    e. Robustness of device
        i. Good / Easy: 2
5. During the 2021 season growers were ask to run multiple spore traps at a single site. Please rate the user experience of running multiple devices at a single site
    a. Rating 5 & 6 out of 10
6. We are keen to make improvements ahead of the next season, please use this space to provide thoughts/recommendations on alterations we could make to the running of the spore trap network ahead of 2022.
    a. Sporestick needs improvement. Especially regarding batteries.
7. Thinking about the sampling protocol, please rate the sampling process against each category
    a. Clarity of instructions

i. Good / Easy: 2

b. Complexity of protocol
    i. Good / Easy: 2
c. Troubleshooting
    i. Poor / Difficult: 1
    ii. Good / Easy: 1
d. Length of sampling season
    i. Good / Easy: 2
e. Sampling frequency and in-season changes
    i. Good / Easy: 2
f. Disease Observations
    i. Poor / Difficult: 1
    ii. Good / Easy: 1

8. So we can improve the process for 2022, we'd like to better understand the challenges growers faced in-field in relation to the follow aspects of the protocol where issues were experienced at some sites/during some periods of the season
    a. Sampling twice per week until spores present at site - Time pressures: 2
    b. Sampling twice per week until spores present at site - Equipment failure: 1
    c. Sampling weekly once spores detected at the site - Time pressures: 1
    d. Sampling weekly once spores detected at the site - Equipment failure: 2
    e. Sampling season concluding end of July - No issues encountered at site manager: 1
    f. Sampling season concluding end of July - Time pressures: 1
    g. Sampling season concluding end of July - Equipment failure: 1
    h. Weekly disease observations - No issues encountered at site manager: 1
    i. Weekly disease observations - Time pressures: 1
    j. Weekly disease observations - Equipment failure: 1
    k. Samples return in unlabelled sample bags / tubes - No issues encountered at site manager: 2
    l. Different number of samples returned vs. protocol - No issues encountered at site manager: 1
    m. Different number of samples returned vs. protocol - Time pressures: 1
    n. Different number of samples returned vs. protocol - Equipment failure: 1
    o. Snapped sampling sticks not containing a full sample - No issues encountered at site manager: 1
    p. Snapped sampling sticks not containing a full sample - Time pressures: 1
    q. Snapped sampling sticks not containing a full sample - Equipment failure: 1

9. General feedback on CropMonitor Pro
    a. Ease of registration
        i. Poor: 1
        ii. Good: 1
    b. Ease of navigation Good: 1
        i. Good: 2
    c. Reliability of service
        i. Good: 2
10. Thinking about the data presented to you on ODM risk on CropMonitor pro - did you use this information for in-field decision making?
    a. Yes: 1
    b. No: 1
11. Thinking about the cube-reading score for samples tested for your site, did you use these results to make decisions in-field?
    a. No: 2
    b. If yes, please describe how you used the cube reading score in your in-field decision making
        i. I didn’t look at them or use them. Not part of my role
12. Please use this space to provide feedback / thoughts on what additional information or changes could be made to the Onions section of CropMonitor Pro to make it a more valuable tool
    a. Difficult to fill in field details.
13. Thinking about Fera's coordination and management of 2021 sampling, please rate each category
    a. Support during the setup of traps
        i. Poor: 1
        ii. Good: 1
    b. Support with troubleshooting trap issues
        i. Poor: 1
        ii. Good: 1
    c. Support with sampling
        i. Poor: 1
        ii. Good: 1
    d. Frequency of updates from project team
        i. Good: 2
    e. Awareness of delivery plan / next steps
        i. Good: 2

f. Further comments
    i. Slow replies and difficult to find solutions to problems.

